Results: Fifty-six patients (31.6%) had elevated cTnI > 1.0 ng/mL at the time of PE diagnosis. Patients with elevated cTnI were older (p<0.01) and had higher incidence of malignancy (p=0.06) but no increased prevalence of prior MI or CAD (p=0.62). Elevated cTnI was assoicated with adverse outcomes including 30-d mortality (p<0.01) (Table 1). After adjusting for possible confounders, the association of cTnI with adverse outcomes remained.

Conclusions: In the setting of acute PE, elevated cTnI is associated with increased 30-d mortality and adverse cardiopulmonary/ hemodynamic outcomes. Elevated cTnI may identify high risk patients with PE more likely to benefit from aggressive therapies such as thrombolysis.

	Troponin I < 1.1 ng/mL (n=121)	Troponin I > 1.0 ng/mL (n=56)	Adjusted RR (95% CI)	P value
Peak cTnI	0.3 ± 0.3	6.3 ± 12.4	-	<0.001
Age (years)	65.7 ± 16.3	71.9 ± 14.0	-	<0.01
Prior MI/Known CAD	25%	21%	-	0.63
30-day Mortality	12%	34%	2.2 (1.5,3.3)	<0.001
Hemodynamcic Compromise	13%	61%	4.5 (2.7,7.3)	<0.001
Mechanical Ventilation	17%	48%	2.6 (1.7,4.1)	<0.001
Pressor Support	9%	36%	2.9 (1.8,4.6)	<0.001

POSTER SESSION

1033 Cardiac Signaling Pathways

Sunday, March 17, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: Noon-1:00 p.m.

1033-90 The Angiotensin II Type I Receptor Associated Protein ATRAP Is a Transmembrane Protein and Negative Modulator of Angiotensin II Signaling

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AT1 receptor-associated protein (ATRAP) was identified by our group in a yeast two-hybrid screen for proteins that bound to the carboxyl-terminal cytoplasmic domain of the Angiotensin II type 1 receptor (AT1). In this work we characterize ATRAP as a transmembrane protein, localized in the endoplasmic reticulum and plasma membrane that functions as a negative modulator of Angiotensin II-induced signal transduction. Endogenous and transfected ATRAP cDNA shows a particulate distribution; hydrophobicity analysis of the primary structure of ATRAP reveals the presence of three transmembrane domains at the amino terminal of the protein and a hydrophilic cytoplasmic carboxyl-terminal tail. Over-expression of ATRAP mutants show that the deletion of the C-terminal domain of ATRAP leads to the formation of prominent perinuclear vesicle clusters, whereas the deletion of the N-terminal transmembrane domains leads to a diffuse cytoplasmic distribution of the protein. Electron microscopy reveals the presence of ATRAP in prominent perinuclear vesicular membranes; colocalization analysis by immunofluorescence show that ATRAP colocalize in the same vesicular compartment of the endoplasmic reticulum marker DiOC6(3). Real time following of ATRAP vesicles shows constitutive translocation towards the plasma membrane. Using epitope tagged forms of ATRAP either at the amino or carboxyl ends of the molecule, we determined the orientation of the amino end as being outside the cell. Functional analysis of the effects of ATRAP on Ang-II induced AT1 receptor signaling reveals a moderate decrease in the generation of inositol lipids, a marked decrease of the Ang II-stimulated transcriptional activity of the c-fos promoter luciferase reporter gene, and decrease in cell proliferation. These results implicate ATRAP as a modulator of AT1 receptor signaling acting in a vesicular compartment, via interactions with the C-terminal domain of the receptor.

1033-91 Mechanisms of Thromboxane A_2 Associated Apoptosis in Adult Cardiac Myocytes: Role of Protein Kinase C Zeta Mediated Downregulation of Akt Activity

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Background: Apoptosis (Apo) is seen in myocardium exposed to acute ischemic insults and Apo may be in part mediated by increased thromboxane A_2 (TXA) level. We investigated the role of protein kinase C (PKC) in Apo induced by TXA. Methods: Adult rat ventricular myocytes (ARVM) were cultured for 48 h before pharmacological interventions. The involvement of PKC was measured with both translocation and immune complex kinase assay. Akt activity was measured with both immunoblotting and immune complex kinase assay. The extent of apoptosis was assessed with TUNEL and DNA ladder assay. Results: Treatment with a TXA mimetic, IBOP for 24 h induced Apo in ARVM in a dose-dependent fashion (35.0±3.0% in 100 nM IBOP vs. 13.9±1.6% in controls, P<0.05, data expressed as %TUNEL positive cells). The Apo by TXA was completely inhibited by a TXA receptor specific inhibitor SQ29548. TXA stimulation resulted in membrane translocation of PKCζ at 3 min and 1 h stimulation, but not PKCα, βII, δ, and ε. The isoenzyme specific activation of PKCζ was confirmed by an immune complex kinase assay. The activation of PKCζ by TXA was also associated with reduction of Akt activity. A cell permeable PKCζ specific pseudosubstrate peptide (ζPS; a gift from Dr. Mochly-Rosen, Stanford University, CA) inhibited apoptosis by TXA at a dose which inhibited TXA-mediated increase in PKCζ activity (1 μM). ζPS also prevented Akt reduction by TXA. The anti-apoptotic effect of ζPS was completely abolished by phosphatidylinositol 3-kinase inhibitors which inhibit ζPS mediated preservation of Akt activity. In addition, moderate PKCζ overexpression for 36 h by adenovirus vector (4.5-fold control) reduced Akt activity to half as well as exaggerated apoptosis by TXA (40.8±3.5% with 100 nM IBOP, P<0.05 vs. 100 nM IBOP alone).

Conclusion: The activation of PKCζ by thromboxane A_2 suppresses Akt activity which in turn, promotes apoptosis in adult cardiac myocytes. Therefore, PKCζ specific inhibition and/or activation of Akt might be useful in preventing thromboxane A_2 mediated cardiac injury.

1033-92 Stimulation of Cyclic AMP Synthesis by Combined Overexpression of the Nucleoside Diphosphate Kinase NM23H2 and the Alpha-Subunit of Gs Proteins

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Background: G proteins are mediators of signalling pathways that are implicated in the development of cardiac hypertrophy and dilation. We have recently suggested a receptor-independent mechanism of G protein activation by a membrane-associated nucleoside diphosphate kinase (NDPK). In sarcolemmal membranes from failing human hearts, the level and activity of NDPK were elevated three-fold, leading to a 50- to 75% inhibition of cAMP-synthesis. This finding could reflect activation of the likewise increased Gi proteins. To prove the interaction of NDPK with a G protein, we transfected cells with genes encoding for NDPK and the alpha-subunit of the stimulatory G protein Gsalpha. Methods: Immortalized cells, derived from neonatal rat hearts, were stably transfected with the NDPK-gene NM23-H2. Overexpression of Gsalpha was induced with a recombinant adenovirus. Intracellular cAMP and adenylyl cyclase activity (AC), NDPK-activity, Gsalpha and Gialpha in membranes were determined. Results: Overexpression of Gsalpha led to an increase in cAMP synthesis in all cell clones, despite the presence of the inverse agonist propranolol. This increase was strictly proportional to the level of NDPK activity in seven different clones. In cells with a 2.7-fold overexpression of NDPK and a 10-fold overexpression of Gsalpha, intracellular cAMP was increased 200-fold compared to control cells. Stable transfection of the cells with a catalytically inactive NDPK did not increase cAMP-synthesis. Intracellular nucleotide concentrations were not influenced by the overexpression of NDPK. In crude membranes from these cells, basal, G protein independent AC activity was similar in all clones. However in cells overexpressing Gsalpha and NDPK, activity in the presence of GDP was increased proportionally to the level of NDPK activity. In conclusion, these results demonstrate for the first time a functional interaction of NDPK with a G protein in an intact cell.

1033-93 Antioxidant Vitamins C and E Administration in Smokers: Effects on Endothelial Function and Serum Levels of Soluble Intercellular Adhesion Molecule-1, Soluble Vascular Cell Adhesion Molecule, and Lipid Hydroperoxides

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Background: Serum levels of soluble vascular cell adhesion molecule (sVCAM-1) and soluble intercellular adhesion molecule (sICAM-1), as well as lipid hydroperoxides (LPO) (marker of lipid peroxidation), are implicated in the pathogenesis of atherosclerosis. Purpose of this study is to investigate the effect of combined administration of antioxidant vitamins C and E on endothelial function, and serum levels of sICAM-1, sVCAM-1 and LPO, in chronic smokers.

Methods: 36 healthy young smokers (20 males 16 females aged 36±2 years) were enrolled in this double blind placebo controlled study. Subjects were divided into 4 groups receiving vitamin C 2g/day (n=10) (group A), vitamin C 2g/day and vitamin E 400IU/day (n=11) (group B), vitamin C 2g/day and vitamin E 800IU/day (n=9) (group C) or placebo (n=6) (group D), for 4 weeks. Forearm blood flow was measured using venous occlusion strain gauge plethysmography. Endothelium dependent flow mediated vasodilation (FMD) was expressed as the % change from baseline to post reactive hyperemia blood flow. Endothelium independent % change flow (NTR%) was assessed after sublingual nitroglycerin administration. Plasma levels of sVCAM1, and sICAM1 were determined by enzyme linked immunosorbent assay, while LPO was determined using a spectrophotometric assay.

Results: Blood pressure, heart rate, body weight, basal forearm blood flow and NTR% were similar before and after treatment. FMD was significantly increased in groups B (46.6±5.5 to 74.1±9.3%, p<0.05) and C (43.6±3.9 to 74.9±4.2%, p<0.01), while remained unaffected in groups A and D. Serum levels of sVCAM-1 and sICAM-1 significantly reduced in group C (from 339±14 and 318±21 to 298±11 and 250±19 ng/ml respectively, p<0.05 for both), while remained unaffected in groups A, B and D. LPO was significantly reduced in groups B and C (from 14.5±1.2 and 15.4±2.9 to 8.8±1.6 and 8.3±1.7 μM respectively, p<0.05 for both).

Conclusions: Chronic administration of vitamin C (2g/day) combined with vitamin E (800IU/day), reduces blood levels of sVCAM-1 and sICAM-1, improves endothelial function and reduces lipid peroxidation in healthy young smokers. These findings may have therapeutic implications in chronic smokers